Reference: *Biol. Bull.*, 136: 454–460. (June, 1969)

THE EFFECT OF PREGNANCY ON PERIPHERAL BLOOD IN THE MOUSE

ROBERTS RUGH [1] AND CSILLA SOMOGYI

Radiological Research Laboratory, Department of Radiology, College of Physicians and Surgeons, Columbia University, New York, New York 10032

The increasing demands by the growing mammalian fetus upon the maternal tissues for food and oxygen would be expected to cause significant changes in the maternal peripheral blood cell counts during pregnancy. So-called "physiological anemia" has been reported in the rat, rabbit, and in man (Zarrow, 1961) but this may be misleading because of the lack of correlation between plasma volume and red cell volumes (Fruhman, 1968). The low hemoglobin levels per unit volume of peripheral blood in human pregnancy is due to a greater increase in total blood volume (30%), plasma volume (40%) than to an increase in red cell mass (18%) (Low, Johnston, and McBride, 1965). Nevertheless peripheral blood changes as reflected in total blood counts associated with the progress of a pregnancy certainly reflect the increasing fetal demands upon the maternal circulation, so that the following study was designed.

Materials and Methods

CF1-S mice used for this study had been matured by being put through a single pregnancy. Thirty mice were time-mated to 45 minutes so that their gestational ages were identical. On each alternate day from day of conception through day 18, fifteen mice were blood checked. Delivery normally occurs on the 19th day after conception. The small amount of blood removed from the tail tip on alternate days was believed to be completely replaced within the 48 hours before the next bleeding. Thus the data given are averages of 15 normal but pregnant mice for each gestation day.

Blood was always taken before noon of the day designated in order to avoid any diurnal shift in the normal counts (Urushijama, 1959). Fetal blood taken on gestation day 17 was removed quickly with pipettes and rubber tubing all ready. The head of the fetus was held in the left hand, the lower part of the abdomen being immobilized by the thumb of the same hand. The jugular vein was plainly visible through the thin fetal skin, and this was pierced with scissors. All counts were based on a minimum of 15 mice of each sex (Rugh and Somogyi, 1968ab).

A complete analysis of the blood smears was made for the 17 gestation day mouse fetus, to compare its varied cells with those found in the maternal peripheral blood. Examination could not be made before the 17th day simply because it was not possible to obtain from earlier fetuses enough whole blood. It was, however, possible to sex mice at 17 days gestation so that the data are divided as to the two

[1] Based upon work performed under Contract AT-(30-1)-2740 for the U. S. Atomic Energy Commission.

sexes and comprise averages of 15 such fetuses. To make a further comparison, blood data were collected from 25 separate 9 week old mature adults of both sexes.

It is known that there are daily rhythms in the various peripheral blood cell types in normal animals so that this variant was avoided by taking all blood samples at the same time of day, namely in the morning (Pauly and Scheving, 1965). There are also variations in blood counts among closely related strains of mice (Russell, Neufeld and Higgins, 1951; Pauly and Scheving, 1965), and even sex differences occur (Halberg, Hammerston and Bittner, 1957). This study was based entirely upon the CF1-S strain, and the sexes were distinguished for all tests including those of the 17 day fetus (Rugh 1968).

Experimental Data

The daily weights were taken of the impregnated mice. The three month old female mice averaged a little less than 25 grams at the time of mating. This

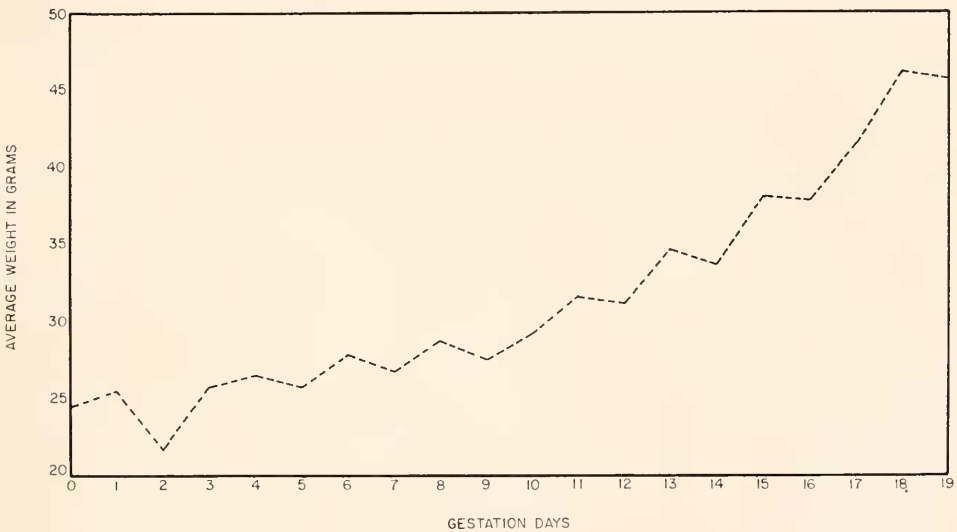

Figure 1. Average weight changes during pregnancy in mice.

average dropped a few grams at the second day. There was then a slow climb in average total body weight when at about 13 days of gestation all mice gave external evidence of their pregnancies and the weight average had increased from about 25 to about 34–35 grams. There followed a steady daily increment in average body weight to a maximum at 18 days (Fig. 1). The average gain in weight before birth was about 22 grams, almost a doubling of the original body weight. This dropped after delivery of the litter to an average total weight of about 31 grams or still some 6–7 grams above the pre-conception weight. This residual weight gain was probably due to mammary activity. Female mice after an initial pregnancy, no matter at what age it occurs, tend to remain heavier than they were prior to that pregnancy.

The average peripheral red blood cell counts of the 25 control 9 week old female mice was 9,700,300 and the initial counts of the impregnated mice was close to this. The accompanying graph shows that there was an immediate but slight drop in the red blood cell count by 1 day after conception, with a further drop developing after day 5 and continuing until just before birth. Maternal blood at parturition was relatively anemic but not seriously so. This anemia might be caused by an avidity on the part of the fetus, since pregnancy in humans is often associated with anemia. The steady drop in red cells began at about 5 days. This is the time when the maternal-fetal relations become quite intimate, following implantation on day $4\frac{1}{2}$, so that decidual hemorrhage may have played some part in the mild maternal anemia (Fig. 2). Maternal blood never becomes seriously anemic since the lowest point, at 17 days, was still about 6,500,000 red cells.

The average white cell counts in the maternal peripheral blood showed two prominent peaks, at 3 and at 9 days. These are probably best explained on the

FIGURE 2. Average red blood cell count changes during pregnancy in mice.

basis of changes in the fetal-maternal relationships. At 3 days the developing morula-blastula stage move through the oviducts into the uteri and as foreign and invading entities they are probably irritants causing some local leukocytosis. This has been observed in sections of the uterus just prior to implantation (Rugh, 1968). It is also possible that the response of the leukocytes to the invasion of the female reproductive tract by sperm cells is relatively slow and the peak of this response seems to persist for about three days after the invasion by the sperm. Such phagocytosis of sperm is regularly seen in post-conception uteri in the mouse. The average white blood cell count of the impregnated mice on conception day was considerably lower than the average for similar, but non-pregnant 9 week old mice and was slightly above 7000. There was a transient but nevertheless real elevation of white cells to about 9800 by 3 days, being the average of 15 mice simultaneously

impregnated. The white cell count then dropped slowly to the 7th day almost to the pre-conception level, but then rose rapidly in two days to another high at 9 days. This second rise could be explained on the basis of placenta formation which begins between 7–8 days and is very active on days 9 and 10. Such an invasion of maternal tissues of the uterus by the fetus, and penetration by fetal villi, could easily cause a rise in the counts of the protective white blood cell elements (Fig. 3). As soon as the placenta was established the white count dropped to its lowest point at 13 days, to remain sub-normal for the duration of the pregnancy. Thus there appears to be a complete adjustment of the maternal blood elements to the invasion by the fetus of the maternal tissues and the white cell count remained considerably below the infection level.

The tabular data from the 17 day old fetus, based upon averages of 15 males and 15 females, is of interest particularly in relation to the peripheral red and the

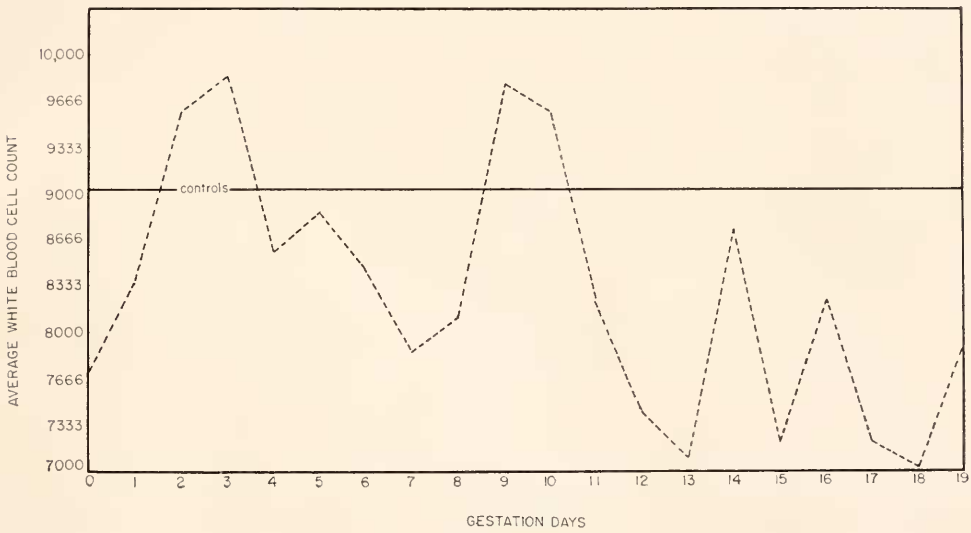

FIGURE 3. Average white blood cell count changes during pregnancy in mice.

white cell counts. These are shown in Table I where the data are also given for the same strain of mice (average of 25 mice for each figure) at 9 weeks of age. It will be noted that the hemoglobin percentage is considerably lower than for the adult controls, and there persist some immature forms. The red cell counts of the 17 day fetuses were about 35% and the white cell counts about 11% that of the adult controls. Platelet counts were also about 33% those of the control adults. Thus, hematopoiesis was not yet adequate to bring the 17 day mouse fetus up to the adult level so that the late mouse fetus must still be dependent upon the mother for oxygen and protection. Among the white cells the lymphocytes appeared to be making greatest proliferative increase at this time, being about 50% of the count found in the adult controls. The neutrophils (except for the stabs) occurred in about the same proportion as in the adults.

TABLE I

Blood analysis of fetal and adult mice

	Fetuses at 17 days		Adults at 9 wks	
	Males (15)	Females (15)	Males (25)	Females (25)
Hemoglobin	8.9	8.3	14.1	15.1
RBC (Erythrocytes)	3,438,666	3,244,000	9,140,800	9,700,300
WBC (Leukocytes)	1,720	1,533	10,520	9,976
Platelets:	453,333	454,000	1,361,600	1,342,000
Neutrophils:Segmented	21.9	25.1	27.1	23.1
Neutrophils:Stabophils	10.3	7.1	1.1	0.4
Neutrophils:Metamyelocytes	1.5	0.8	0.0	0.0
Neutrophils:Myelocytes	0.1	0.1	0.0	0.0
Eosinophils:	2.5	1.0	3.5	4.1
Eosinophils:Meta	0.5	0.0	0.0	0.0
Monocytes:	18.7	19.7	5.8	4.3
Monocytes:Young	0.9	1.1	0.0	0.0
Lymphocytes:	31.8	30.4	61.5	67.0
Lymphocytes:Young	2.1	2.4	0.2	0.1
Blasts:	0.5	0.8	0.0	0.0
Histiocytes:	0.3	0.1	0.0	0.0
Phagocytes:	0.4	0.7	0.0	0.0
Granular-Vacuolated Cells:	0.9	4.1	0.0	0.0
Early Type Ring Nuclear Cell:	5.3	4.6	0.04	0.1
Double Nucleated Lymphocytes:	0.1	0.1	0.5	0.5
Nucleated Erythrocytes:	48.6	53.7	0.0	0.0
Unidentifiable Cells:	2.0	0.4	0.0	0.0

Young Lymphocytes: stage between blast and adult (one without nucleoli and less blue cytoplasm)

Histiocytes: a very early cell with no phagocytized particle but less granules.

DISCUSSION

Accelerated erythropoiesis does occur in the pregnant mouse (Fowler and Nash, 1967), but in the fetus the first blood cells to appear do so at about 7 days (Rugh, 1968). There is evidence of the erythropoietic initiation independent of the mother (Jacobson, Marks and Gaston, 1959). The yolk sac hematopoiesis provides the primitive generation of erythrocytes which are exceptionally large, nucleated cells. As early as 13 days gestation the nucleated erythrocytes begin to disappear, but as late as 17 days some of these miniature red cells may be seen. The liver, spleen, and bone marrow begin their active hematopoiesis when the circulatory system is closed and functioning by 9 days gestation. Thus, the fetus, independently of the mother, rapidly forms its own circulatory and hematopoietic system, with myelopoiesis starting about day 15.

While mouse development is telescoped into about 19 days, there is relatively little involvement of the maternal tissues until implantation (about day $4\frac{1}{2}$). The sudden and drastic rise in the white count at 3 days can be explained only on the basis of a defense reaction by the maternal tissues against the invasion and irritation of the genital tract by the relatively high volume of semen and spermatozoa. Histological sections of the uterus at this time show massive migration of leukocytes

toward the lumen, and actual phagocytosis of the spermatozoa so that relatively few of them survive to reach the ampulla for fertilization.

A recent study (Fruhman, 1968) indicated that the maternal spleen is the primary organ of erythropoiesis in the mouse, and it was this organ that showed the most striking changes during pregnancy. The reason for such changes in the spleen was believed to be the increasing oxygen requirements of the fetal tissues. Since litter numbers vary from 1 to about 20, it is simple to imagine that the degree of tissue hypoxia may be varied in direct relation to the numbers of viable fetuses. As the fetuses increase in total volume this increased hypoxia could readily affect both fetal and the maternal hematopoietic organs. After the 5th day of gestation the red cell counts dropped steadily reaching a low just before delivery, so that both mother and fetus were anemic. This suggests that the maternal hematopoietic organs did not respond to hypoxia by accelerated hematopoiesis but that the mother's red cell counts changed toward the level of anemia while the fetus was accelerating hematopoiesis. The mother showed a steep incline toward recovery in red blood cells as early as 18 days, when the fetus normally begins to become functionally independent. Blood volume studies were not made. Hypoxia, therefore, did not stimulate maternal hematopoiesis but may be related to fetal hematopoiesis.

Summary

1. Fifteen pregnant mice, time-mated so as to be simultaneously at the same stages of gestation, were weighed daily at the same time for the duration of their pregnancies. As expected, except for gestation day 2, there was a steady increment to 45 grams in the average total body weight of the 15 pregnant mice at day 18, as the fetuses are preparing for birth. The average total body weight almost doubled the original body weight of 25 grams.

2. The white blood cell counts of the pregnant mouse showed a rapid rise by the third day, possibly related to marshalling of leukocytes (neutrophils and macrophages) to phagocytize the invading spermatozoa, or to changes in the uterine mucosa anticipating implantation which usually occurs at day 4½. There is a second peak in the peripheral white cell count at 9 days when the placenta is being organized. Thereafter the average white cell count remains below the normal level for this strain of mouse, with a slight rise of the average at 14 days and another at 16 days, but neither peak bringing the count up to normal.

3. The maternal erythrocyte count showed a steady decline almost from the day of conception, approaching the level of anemia but indicating possibly that the growing fetuses were depriving the mother of nutritive elements necessary for erythropoiesis.

4. The fetal blood at 17 days gestation, two days before birth, showed average deficiencies in the hemoglobin, red and white cell counts and in platelets. Adult type lymphocytes were about 50% of the adults and nucleated reds were still abundant.

5. In general there was evidence of a decreasing average of peripheral erythrocytes in the blood of pregnant mice as gestation progressed while the white cells showed several peaks of production probably related to major structural and functional changes of the intimate relation between fetus and mother. These struc-

tural changes related to implantation, placentation, and the functional changes to increasing hypoxia in the crowded condition of the gravid uterus.

LITERATURE CITED

Fowler, J. H. and D. Nash, 1967. Erythropoiesis in the pregnant mouse. *Exp. Hematol.* **12**: 72–73.

Fruhman, G. J., 1968. Blood formation in the pregnant mouse. *Blood J. Hematol.* **31**: 242–248.

Halberg, F., O. Hamerston and J. J. Bittner. Sex difference in eosinophil counts in tail blood of mature B_1 mice. *Science,* **125**: 73.

Jacobson, L., E. K. Marks and E. O. Gaston, 1959. Studies in erythropoiesis. XII The effect of transfusion-induced polycythemia in the mother on the fetus. *Blood,* **14**: 644–653.

Low, J. A., E. E. Johnston and R. L. McBride, 1965. Blood volume adjustments in the normal obstetric patient with particular reference to the third trimester of pregnancy. *Amer. J. Obstet. Gynecol.* **91**: 356–363.

Pauly, J. E. and Scheving, 1965. Daily leucocyte rhythms in normal and hypophysectomized rats exposed to different environmental light-dark schedules. *Anat. Rec.* **153**: 349–360.

Rugh, R., 1968. *The Mouse, Its Reproduction and Development.* Burgess Pub. Co., Minneapolis, Minnesota, 430 pp.

Rugh, R. and C. Somogyi, 1968a. Pre- and post-natal mouse blood cell counts. *Proc. Soc. Exp. Biol. Med.,* **127**: 1267–71.

Rugh, R. and C. Somogyi, 1968b. Hematological recovery of mouse following fetal x-irradiation. *Biol. Bull.,* **134**: 320–324.

Russell, E. S., E. F. Neufeld and C. T. Higgins, 1951. Comparison of normal blood picture of young adults from 18 inbred strains of mice. *Proc. Soc. Exp. Biol. Med.,* **78**: 761–766.

Urshiyama, Y., 1959. Diurnal fluctuation of leucocyte counts of subjects with radiation hazards. *Nippon Igaku Haskesen Gakkai Zasski,* **19**: 1333–45.

Zarrow, M. X., 1961. Gestation, pp. 958–1031. *In:* Edgar Allen, Ed., *Sex and Internal Secretion, Volume II.* Williams and Wilkins Co., Baltimore.